

Aim 13

To separate the Plant pigments by Paper Chromatography

Introduction

A technique which is used for the separation of molecular component from the mixture by using some absorbents such as gel and paper is termed as chromatography. Mobile phase contains the sample mixture. When carried through mobile phase on chromatographic paper, the interaction between stationary and mobile phase is observed.

Various component of sample mixture extract can be differentiated on the basis of small difference in their physiochemical properties. The compounds show rapid migration which interacts more with mobile phase. The differential movement is an ultimate reason for the separation of the compounds.

For separation and purification of plant pigments (Chl a, Chl b and Carotenoids) amino acids from the mixture, use paper chromatography by using Whatman's filter paper no. 1.

R_f value of separation compound is calculated after complete separation and then compared with the standard value for identification.

Requirements

Leaves of *Moringa*, petroleum ether, acetone, spirit lamp, pestle mortar, beakers, cylinder, muslin cloth, capillary cloth, chromatographic paper and dryer.

Formula Used

$$R_f \text{ (retention factor)} = \frac{\text{Distance travelled by solute from the loading point}}{\text{Distance travelled by solvent from the loading point}}$$

Preparation of extract:

1. Some leaves of *Moringa* are taken and wash them properly with distilled water.
2. Use pestle and mortar for mashing the leaves in a minimum amount of acetone.
3. Filter the above with muslin cloth.
4. Discard the residue and filtrate is used for loading.

Preparation of running solvents:

It is prepared by mixing the petroleum ether and acetone in 9:1 ratio.

Procedure

1. A chromatographic paper is taken and draw a line at 2.5 cm above from the bottom with pencil, mark a small circle of about 3 mm diameter in the centre of this line.
2. A fine glass jet is prepared by heating the capillary tube over the flame. Use this jet and load the plant extract inside the small circle. Use dryer to dry it. This process is repeated 5-8 times.
3. After loading, put the strip in measuring cylinder, having running solvent.
4. Hang the strip in such a way that, the lower portion of the strip is dipped in the solvent while the spot is outside.
5. Leave the cylinder undisturbed for hours.
6. After that take out the strip and dry it.
7. Various pigments are recognized on the basis of the colour and pencil is used to mark them.